Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo **Agar Gelidium** [redacted] No Date

PROTOCOL

1K

Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

AGAR GELIDIUM

Protocol:

Agar gelidium was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by the two routes at two stages of embryonic development: via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (1, 2).

Groups of 10 or more eggs were treated under these four conditions at several dose levels until a total of ninety to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. All hatched chicks and non-viable embryos were examined carefully for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively (the milligrams per kilogram figure is based on an average egg weight of fifty grams). Column 3 is the total

number of eggs treated. Column 4 is the percent mortality i.e. total non-viable divided by total treated eggs. Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders. Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent treated eggs and the untreated controls.

The mortality data in Column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (3). The results obtained are indicated at the bottom of each table.

The data of Columns 4, 5, and 6 have been analyzed using the Chi Square Test for significant differences from the control background. Each dose level is compared to the control value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

At hatchings, 3 chicks were removed at random from each level including control for skeletal clearing, weighing and fixing of bursa, spleen, liver and kidney. Tissues were processed, blocked in paraffin, sectioned, affixed to slides, and stained. Later these sections were examined for internal damage to the tissues.

Discussion:

Agar Gelidium was tested at dose levels between 2 and 10 mg/kg in all the test conditions employed. The estimated LD-50 levels for four different treatments used in the test were as follows:

Treatment	LD-50 Level
Air cell treatment 0 hours	13.46 mg/kg (0.67 mg/egg)
Air cell treatment 96 hours	11.14 mg/kg (0.56 mg/egg)
Yolk treatment 0 hours	5.48 mg/kg (0.27 mg/egg)
Yolk treatment 96 hours	6.62 mg/kg (0.33 mg/egg)

Agar gelidium when administered through air cell 6 mg/kg dose produced significantly high mortality rate. However, at 96 hours of embryonic treatment 6 mg/kg was not severe. In yolk treatment, the effects of agar gelidium on percent mortality were more severe. At 0 hours of yolk treatment, 2 mg/kg dose was tolerable, but at 96 hours of embryonic treatment even that small dose of 2 mg/kg produced significantly different percent mortality rates.

Agar gelidium had no observable teratogenic effects on embryos under the test conditions employed.

References:

1. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

2. Verrett, M. J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1003-1006.

3. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendic I.

AGAR GELIDIUM
AIR CELL AT 0 HOURS

DOSE		Number of Eggs	Percent Mortality *	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.50	10.00	100	43.00*	0	0
0.40	8.00	100	38.00*	0	0
0.30	6.00	100	27.00*	0	0
0.20	4.00	100	18.00	0	0
0.10	2.00	100	12.00	0	0
Water		100	11.00	0	0

*Significantly different from solvent $p \leq 0.05$

AGAR GELIDIUM
AIR CELL AT 96 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.50	10.00	100	50.00*	0	0
0.40	8.00	100	38.00*	0	0
0.30	6.00	100	26.00	0	0
0.20	4.00	100	14.00	0	0
0.10	2.00	100	11.00	0	0
Water		100	14.00	0	0

*Significantly different from solvent $p \leq 0.05$

AGAR GELIDIUM
YOLK 0 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.50	10.00	100	83.00*	0.0	0.0
0.40	8.00	100	73.00*	0.0	0.0
0.30	6.00	100	65.00*	0.0	0.0
0.20	4.00	100	61.00*	0.0	0.0
0.10	2.00	100	47.00	0.0	0.0
Water		100	33.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

AGAR GELIDIUM
YOLK 96 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.50	10.00	100	80.00*	0.0	0.0
0.40	8.00	100	70.00*	0.0	0.0
0.30	6.00	100	55.00*	0.0	0.0
0.20	4.00	100	54.00*	0.0	0.0
0.10	2.00	100	47.00*	0.0	0.0
Water		100	31.30	0.0	0.0

*Significantly different from solvent $p \leq 0.05$